Enzyme-Linked Immunosorbent Assay for Detection of Antibodies to *Anaplasma centrale*

Yoshio NAKAMURA, Shinya SHIMIZU, Tetsuro MINAMI, and Shingo ITO

National Institute of Animal Health, 3–1–1, Kannondai, Tsukuba, Ibaraki 305, Japan

(Received 28 January 1988/Accepted 14 April 1988)


---

**KEY WORDS:** *Anaplasma centrale*, cattle, ELISA.

The intraerythrocytic rickettsia *Anaplasma centrale* has been detected from grazing cattle in many pasture in Japan [1]. *A. centrale* is considered to be rather aapathogenic, however, mixed infection of *A. centrale* with *Theileria sergenti* and/or *Babesia owa* is one of the conceivable cause to bring anemia on cattle [5]. This paper describes the application of enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to *A. centrale*.

The Aomori isolate of *A. centrale* [1] was used in this study. A splenectomized Holstein calf was subcutaneously inoculated with 10⁸ parasitized bovine erythrocytes. On day 35 after inoculation, 1 l of blood from the calf (number of erythrocytes; 6.4×10⁹/mm³, parasitemia of *A. centrale*; 10.0%) was intravenously injected into next splenectomized calf. The second calf was bled on day 14 (number of erythrocytes; 7.3×10⁹/mm³, parasitemia; 33.1%). *A. centrale* bodies were collected from 5 l of blood by means of the modified nitrogen decompression method described previously [4], and 4 ml of *A. centrale* pellet was obtained. The pellet was suspended in 20 ml of phosphate buffered saline (PBS, pH 7.2), and further disrupted by sonication (35 watts, 3 min). The suspension (protein concentration; 2.2 mg/ml) was incubated with equal quantity of 4% solution of Triton X-100 in PBS for 3 hr at 4°C. After removing the nonsolubilized components by centrifugation, the supernatant was used as the ELISA antigen.

The ELISA reaction was performed as described previously [4]. The horseradish peroxidase conjugated rabbit IgG fraction anti bovine IgG (Cappel Lab. Inc., Worthington, Minnesota) was diluted to 1:10,000. The complement fixation test (CFT) was performed by the method described previously [3] using the sonicated *A. centrale* suspension as CFT antigen after diluting to 1:32 with veronal buffer (pH 7.3). Blood film samples were stained with Giemsa solution and the parasitemias were calculated.

ELISA antigen was diluted from 1:2,000 to 1:8,000 and titrated against reference sera to determine the optimal dilution of ELISA antigen. The positive reference serum (PS, CF titer=1:40) was obtained from the calf experimentally infected with *A. centrale* and the negative reference serum (NS, CF titer=<1:5) from a normal calf. The result indicated that optical density at 490 nm (OD₄₉₀) was about 1.0 in the reaction against PS when the antigen dilution was 1:3,000. The OD₄₉₀ was under 0.07 in the reaction against NS. In the following tests, ELISA antigen was diluted to 1:3,000, and the results were given an ELISA value corresponding to the ratio of OD₄₉₀ of serum sample against that of PS.

To examine the specificity of ELISA antigen, serum samples were collected from 101 normal calves and five calves each experimentally infected with *A. centrale*, *A. marginale*, *B. bigemina*, *B. bovis*, *B. owa*, *Eperythrozoon wenyonii* and *T. sergenti*. Antisera to *A. centrale* and *A. marginale* were also evaluated ELISA values using *A. marginale* antigen as described before [4]. The average ELISA value of normal sera measured using *A. centrale* antigen was 0.07±0.034 (mean±S.D.). ELISA reaction was considered positive when ELISA value was higher than 0.16 (the upper limit of 99% confidence interval). As shown in Table 1, four of five antisera to *A. marginale* had positive ELISA values in the reactions using *A. centrale* antigen. All of them, however, were demonstrated higher ELISA values in the reactions using *A. marginale* antigen. The ELISA values of antiserum to *Babesia*, *Eperythrozoon* and *Theileria*, were all under 0.11.

To compare the antibody response measured by ELISA and CFT, two splenectomized calves were subcutaneously inoculated with 10⁸ parasitized bovine erythrocytes. Serum samples were obtained at 2 to 5-day intervals. As shown in Fig. 1, ELISA values increased to the positive levels in earlier stages of infections (calf No. 1; 2 weeks, calf No. 2; 8 weeks after inoculation) than the CF titers turned out 1:5 and *A. centrale* bodies first appeared in the erythrocytes.
Table 1. Cross reactions of A. centrale and A. marginale

<table>
<thead>
<tr>
<th>antiserum to</th>
<th>ELISA value measured with</th>
<th>antiserum to</th>
<th>ELISA value measured with</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. centrale</td>
<td>A. centrale antigen (^b)</td>
<td>A. marginale antigen (^b)</td>
<td>A. marginale antigen (^b)</td>
</tr>
<tr>
<td>No.1</td>
<td>0.66</td>
<td>0.21</td>
<td>No.1</td>
</tr>
<tr>
<td>No.2</td>
<td>0.89</td>
<td>0.37</td>
<td>No.2</td>
</tr>
<tr>
<td>No.3</td>
<td>0.96</td>
<td>0.45</td>
<td>No.3</td>
</tr>
<tr>
<td>No.4</td>
<td>1.00</td>
<td>0.32</td>
<td>No.4</td>
</tr>
<tr>
<td>No.5</td>
<td>1.01</td>
<td>0.65</td>
<td>No.5</td>
</tr>
<tr>
<td>AC-PS (^c)</td>
<td>1.00</td>
<td>0.58</td>
<td>AM-PS (^d)</td>
</tr>
</tbody>
</table>

a) Positive ELISA value is higher than 0.16.
b) Positive ELISA value is higher than 0.20 [4].
c) Positive reference serum for A. centrale ELISA.
d) Positive reference serum for A. marginale ELISA [4].

Fig. 1. Antibody response of the calves experimentally infected with A. centrale.

It was described previously that A. marginale antigen solubilized with Triton X-100 was suitable for the ELISA to detect antibodies to A. marginale [4]. This study demonstrated that the ELISA utilizing A. centrale antigen prepared as the same method was suitable for the screening of A. centrale infection from the following reasons; ① the required amount of antigen protein was only 37 ng in each microplate well, ② it was more sensitive than the CFT, ③ its simple procedure would allow large scale screening.

It is known that A. centrale and A. marginale mutually cross react in CFT [2], capillary tube agglutination test [6] and indirect fluorescent antibody test [7]. The cross reaction in this ELISA was not unexpected. Both antisera to A. centrale and those to A. marginale indicated higher ELISA values using the homologous antigen than using the heterologous one. It should be possible to distinguish the infections with A. centrale and A. marginale by ELISA utilizing both Anaplasma antigens.

REFERENCES

要約

酵素抗体法を用いた Anaplasma centrale 感染牛の抗体検出（短報）：中村義男・清水真也・南哲郎・伊藤進午（農林水産省家畜衛生試験場）——酵素抗体法（ELISA）による Anaplasma centrale 抗体の検出を試みた。本法は感度が高く、実験感染牛では補体結合反応より早期から感染抗体を検出することが可能であった。A. marginale 感染牛血清とは交差反応を示したが、A. centrale, A. marginale 感染牛血清のいずれもヘテロの抗原との反応に比べてホモの抗原との反応でより高い ELISA 値を示した。